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Genotyping of Escherichia Coli Isolated From Clinical and Hospitals Environment

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Abstract

Pathogenic *Escherichia coli* (*E. coli*) strains are known to cause intestinal and extraintestinal infections in human. A wide variety of infectious diseases could be caused by *E. coli* strains, including urinary tract infection, septicemia, newborn meningitis, central nervous system and respiratory system infections.Several bacterial agents can cause diarrheal and urinary tract infections, among these *E.coli* strains are detected as an important cause of morbidity and mortality of diarrhoea and UTI throughout the world.A total of 318 samples were collected during the period from September 2012 to march 2013 .All specimens were screened for the presence of *E. coli* by cultured on MacConkey agar and Eosin-methylene blue agar then identified by biochemical tests and confirmed by API 20E system which revealed that:90/318(28.33%) gave positive growth for *E.coli* (15 (16.63%) environmental isolates and 75(83.33%) clinical isolates) as fallowing:1/42(2.38%)was obtained from burns,7/25(28%)from High vaginal swabs ,11/41 (26.82%) from Wounds infections,18/33(54.54%)from Diarrheal infections and38/67(56.71 %)from Urinary tract infections, while 15/110 (13.63%) from environmental isolates, The isolated were subjected to molecular identification as *E.coli* by used the intergenic spacer region primer(*ITS*). The confirmed isolates were examined to detect the phylogenetic group based on triplex PCR by using a combination of two genes (*chuA* and *yjaA*) and an anonymous DNA fragment.Universal primers (*ITS*) indicated that all isolates 90(100%) gave positive results.The phylogenetic analysis revealed that 90 *E.coli* isolates belonged to three phylogenetic groups A &D 36(40%)for each, while B2 18 (20%) and no one isolates belong to B1 phylogenetic types .Out of the 16 of antibiotics used, 87(92.22%) isolates were multidrug resistance.All isolates have been found resistant to at least one β-lactam Antibiotics.

Key words: *Escherichia coli*, Molecular identification, Genotyping

المستخلص

السلالات المرضيه للاشريكية القولونية تسبب للإنسان اصابات داخل وخارج الامعاء. وهي مسوؤلة عن طيف واسع من الامراض المعدية منها اصابات المجاري البولية ،تسمم الدم ،التهاب السحايا لحديثي الولادة وكذلك اصابات الجهاز العصبي المركزي و الجهاز التنفسي . من بين العديد من البكتريا المسببة لإصابات الاسهال والمجاري البولية تعد الاشريكية القولونية واحدة من اهم تلك المسببات جمعت حوالي٣١٨ عينة خلال الفترة من ايلول ٢٠١٢ ولغاية اذار ٢٠١٣. أظهرت نتائج الزرع الأولي على وسط أكار الماكونكي واكار الايوسين مثيلين الازرق بعد التشخيص بالاعتماد على الصفات الزرعية والكيموحيوية وتوكيد التشخيص بأستعمال العدة AP١٢٠E .تبين ان ٩٠ عينة من اصل ٣١٨ عينة اعطت نمو موجبا لبكتريا الاشريكيه(١٥عينة بيئية و٧٥عينة سريرية) وكما يلي : ٤٢/١(٢.٣٨%)من الحروق،٢٥/٧(٢٨%)من المسحات المهبلية،٤١/١١ (٢٦.٨٢%)من حالات الجروح ، ٣٣/١٨(٥٤.٥٤%)من اصابات الاسهال و ٦٧/٣٨(٥٦.٧١%)من التهابات المجاري البولية في حين جمعت ١٥ /١١٠(١٣.٦٣ %)من بيئة المستشفيات. ابدت جميع العينات(٩٠) نتيجه موجبه تجاه التشخيص الجزيئي بأستعمال البادئة (*ITS*).ثم فحصت بعد ذلك لكشف عن انماطهاالجينية بأستخدام البوادئ(*chuA,yjaA,TSP*).اذ توزعت العزلات الى ثلاث مجاميع جزيئية المجموعةAو D (٤٠ %)، B٢ (٢٠%)بينما لم تنتمي أي من العينات الى المجموعة B1.أظهرت٨٧ (٩٢.٢٢ %)عزلة مقاومة متعددة لمضادات الحيويه بعد استخدام ١٦ نوع من المضادات.كما و اظهرت جميع العزل مقاومه لنوع واحد على الاقل من مضادات البيتا لاكتام.

الكلمات المفتاحية :الاشريكياالقولونية،التشخيص الجزيئي،التنميطالجيني.

Introduction

Escherichia coli was first identified by the German pediatricianTheodore Escherich in 1885 during his studies of the intestinal flora of infants, *E. coli* are common inhabitants of the small intestine and large intestine of mammal(Hooper & Gordon, 2001).They can occasionally be isolated in association with the intestinal tract of no mammalian animals and insects. The presence of *E.coli* in the environment is usually considered to reflect fecal contamination and not the ability to replicate freely outside the intestine. There is evidence however to suggest that *E. coli* may freely replicate in tropical fresh water (Baylis *et al.*, 2006). *E.coli* is gram- negative, non–spore forming bacilli; they are approximately 0.5 µm diameter and 1.0–3.0µm in length, within the periplasm is a single layer of peptidoglycan (Eisenstein, 1987). *E. coli* is usually motile in liquid by a set of peritrichous flagella, and also has fimbriae (pili) or fibrillar proteins often extending in great numbers from the bacterial surface and far out into the surrounding medium (Brenner *et al*., 2004). One of the traits commonly encoded on the larger genetic islands of the different pathotypes of *E.coli* are additional pili (chaperone-usher and type IV pili families and non-pili adhesions)(Schreiber &Donnenberg, 2002).In some strains, the outer membrane of *E.coli* is covered by a polysaccharide capsule composed of K antigens. In other polysaccharides, the M antigens are synthesized under conditions of high osmolarity, low temperature, and low humidity (Schaechter, 2009). *E. coli* is a facultative anaerobe. It is capable of reducing nitrates to nitrites. When growing fermentatively on glucose or other carbohydrates, it produces acid and gas (mainly H_2 and CO_2). by traditional clinical laboratory biochemical tests ,*E. coli* is positive for indol production and methyl red .Most strains are oxidase ,citrate, urease , and hydrogen sulfide negative(Harley and Prescott,1996) .Most *E.coli* strains are capable of growing over a wide range of temperature approximately (15-48ºC),the growth rate is maximal in the narrow range of (37º-42ºC) ,it is can grow within a pH range of approximately(5.5–8.0) with best growth occurring at neutrality. Some diarrheagenic *E.coli* strains have the ability to tolerate exposure to pH 2.0. Such an acid shock mimics transit through the stomach and induces expression of sets of genes involved in survival and pathogenesis(Braun and Braun,2002).Clinically, two distinct types of pathogenic *E. coli* are recognized ;One group commonly called extraintestinal pathogenic *E. coli* (ExPEC) includes those *E. coli* associated with newborn meningitis (NBM) or sepsis and urinary tract infections (UTIs) (Johnson *et al*., 2001).Several virulence factors (VFs) enhance the capacity of *E. coli* to cause systemic infections; unlike most commensal *E.coli* strains, extraintestinal isolates possess genes encoding putative VFs of extraintestinal pathogenic *E.coli*(ExPEC),which include various combinations of adhesins (P and S fimbriae),toxins(e.g., hemolysin and cytonecrotizing factor),polysaccharide coatings including (capsules and lipopolysaccharides), siderophores or iron acquisition systems (e.g.,aerobactin and yersiniabactin), serum resistance mechanisms, and invasins ,These VFs help the organisms to colonize host surfaces, avoid and/or subvert host defense mechanisms, injure and/or invade host cells and tissues, and incite a noxious inflammatory response, thereby leading to clinical disease(Bingen-Bidois *et al* .,2002) . The second group termed intestinal pathogenic *E.coli* (IPEC) includes *E.coli* responsible for a range of distinct classes of diarrhoeal disease .Six distinct groups have been defined within IPEC commonly associated with intestinal disease: enteroinvasive *E.coli* (EIEC), enterotoxigenic *E.coli* (ETEC) ,enteropathogenic *E.coli* (EPEC),enterohaemorrhgic *E.coli* (EHEC) enteroaggregative *E. coli* (EAEC) and the diffusely adherent *E.coli* (DAEC)(Donnenberg, 2002; Kaper *et al*,2004). *E.coli* serotypes defined by serum antibodies of the patient or animals that identify the specific type of antigen presented by bacteria. There are three major surface antigens that enable serotyping of *E.coli*. the "O"antigen, are somatic cell wall phospholipid-polysaccharide complex ,the "H" antigens are components of flagella, and "K" antigens, are the surface or capsular antigens that are acidic polysaccharides(Doyle *et al*.,1997).Over 700 antigenic types (serotypes) of *E.coli* are recognized. There are 173 known somatic (O),80 capsular (K),and 56 flagellar (H) antigens, many of which can be subdivided into partial antigens(Rivera and Keen, 2001; Walter and Stamm, 2006).Evolutionary studies based on either DNA sequence analysis or multilocus enzyme electrophoresis has identified clonal phylogenetic groupings of *E. coli*. Phylogenetic studies have principally used the *E. coli* reference (ECOR) strain collection as a common reference for current evolutionary comparisons(Ochman &Selander, 1984).An alternative approach relying on the phylogenetic grouping of E. coli strains based on genetic markers, chuA, yjaA, and TspE4.C2 DNA

fragment, was recently studied in application for microbial source tracking (MST). Based on the study by Clermont et al(2000) and recently ,Carlos et al (2010).E. coli can be categorized into four phylogenetic groups: A, B1, B2, or D, and then into subgroups: A0, A1, B1, B22, B23, D1, and D2. The groups were determined based on the presence or absence of chuA, yjaA, and DNA fragment TspE4.C2. chuA is known to be involved with heme transport while yjaA is involved in cellular response to hydrogen peroxide and acid stress, The function of TspE4.C2 is not yet discovered. E. coli containing combinations of one, two, all, or none of these genes were categorized into seven subgroups(Cherry,2011). Pathogenic E. coli strains derive mainly from phylogenetic group B2 and to a lesser extent group D (Ewers et al., 2007). Commensal E. coli by contrast are characteristically from phylogenetic groups A and B1(Lecointre et al., 1998; Johnson and Stell, 2000).The study was aimed to phylogeny of Escherichia coli isolated from clinical specimens (Burns,wounds , urinary tract infections , diarrhea , and vaginal swabs) and hospitals environments and determination their antimicrobial susceptibility.

Materials And Methods

Samples: samples were collected during the period from September 2012 to March 2013. A total of 318 samples (25 High vaginal swabs, 33 Diarrheal specimen, 41 Wounds swabs,42 Burns swabs,67 Urinary tract infections and 110 environmental) were collected from patients retened /or admitted to two (Al hussein Teaching Hospital and Bint Alhuda Teaching Hospital) hospitals and Public Health Laboratory in Thi-Qar province . All samples were collected by under sterile condition.

Isolation and Identification of Bacterial Isolates

All specimens were cultured on MacConkey agar and Eosin Methylene Blue agar plates ,They were incubated overnight at 37°C in bacteriological incubetors under aerobic conditions.Then typical lactose fermenter colonies on MacConky plates were picked off and transferred to new MacConkey agar plates for additionally overnight, Depending on morphological features of the colonies and microscopically examination with Gram's stain, The pure cultures were prepared for classical biochemical tests to differentiate *E.coli* from other *Enterobacteriaceae* depending on six biochemical tests (positive for methyl red and indole tests, negative for the Voges-Proskauer, Simmon citrate, and urease tests), acid/acid with gas production in the Kliglers iron agar test(MacFaddin, 2000).The diagnosis of 90 E.coli isolates were confirmed by using API 20E system.

Antibiotic susceptibility

Testing susceptibility of 90 isolates against 16 antimicrobial agent (Bioanalyse, Turkey) from different classes has been determined using Kirby-Bauer disk diffusion method (Bauer *et al*.,1966).Inhibition zone around the antibiotic disk was measured according to CLSI(2007).

Polymerase Chain Reaction Assay

1- Preparing the Primers

The Alpha DNA primers were prepared depending on manufacturer instruction by dissolving the lyophilized product with sterile deionized water after spinning down briefly. Working primer tube was prepared by diluted with deionized D.W. The final picomoles depended on the procedure of each primer.

2 -E. coli DNA Extraction

This procedure was done by using commercially available Genomic DNA Mini kit (Blood/culture cell)(Geneaid , UKAS).The procedure was explained in details in user's manul.

3-PCR Supplies Assembling and Thermocycling Conditions

E. coli DNA templates were subjected to PCR using 4 sets (F and R) of primers to molecular identifies and determines *E.coli* phylogeny (Table 1). The PCR reaction mixture contained 20 µL of AccuPower® PCR PreMix each product line contains (1U *Top* DNA polymerase, 250µM dNTPas, 10 mM Tris-HCL(pH 9.0), 30 Mm KCl_2 ,1.5 Mm $MgCl_2$, stabilizer and tracking dye),2µl of DNA tamplete, 1µl of each forward and reverse primers, then the volume completed to 20 µl by deionized D.W. All tubes then shacked well by vortex for 30 seconds. All materials were mixed in same PCR tube on ice bag under sterile condition. The PCR amplification conditions performed by using tow protocols depending on manufacturer's instruction(Table 2). The PCR amplification product was visualized by electrophoresis on 1.4% agarose gels for 45 min at 70 V. The size of the amplicons was determined by comparison to the 100 bp allelic ladder(Bioneer ,South korea).

Table (1): Intergenic spacer primers for ARISA analysis and *E. coli* ABD typing

Primer Name		DNA Sequences (5'-3')	Product Size bp	Reference
ITS	F	GTCGTAACAAGGTAGCCGTA	28-1558	(Cardinale *et al.*,2004)
	R	GCCAAGGCATCCACC		
chuA	F	CGGACGAACCAACGGTCAGGAT	281	(Kotlowski *et al.*, 2007)
	R	TGCCGCCAGTACCAAAGACACG		
Yja	F	CGTGAAGTGTCAGGAGACGCTGC	226	(Kotlowski *et al.*, 2007)
	R	TGCGTTCCTCAACCTGTGACAAACC		
Tsp	F	GGGAGTAATGTCGGGGCATTCAG	161	(Kotlowski *et al.*, 2007)
	R	CATCGCGCCAACAAAGTATTACGCAG		

Table (2): Programs of PCR thermocycling condition

Monoplex gene	Temperature (^{o}C)/ Time				
	Initial denaturation	Cycling condition (30 Cycles)			Final extension
		denaturation	annealing	extension	
ITS	94/3min	94/45 sec	55/1 min	72/2min	72/7 min
Muliplex gene					
ChuA Yja A TspE4.C2	94/ 4 min	94/45 sec	59/1min	72/ 90 sec	72/ 5min

Table (3): Numbers and sources of environmental samples collected during the study

Source	No. of samples	*E.coli* No.	%
Operation hall	11	2	(18.18)
medical equipment	11	2	(18.18)
Birth auditorium	11	2	(18.18)
Burns unit	11	-	(0)
Wards	11	1	(9.09)
Floor	11	1	(9.09)
Beds	11	3	(27.27)
Tables	11	1	(9.09)
Walls	11	-	(0)
Washing basins	11	3	(27.27)
Total	110	15	

Table (4): The number and percentage of clinical samples were collected in the present study

Sources of samples	No. of Samples	*E. coli* No.	%
Burns swabs	42	1	(2.38)
Urinary tract infections	67	38	(56.71)
Diarrheal specimen	33	18	(54.54)
Wounds swabs	41	11	(26.82)
High vaginal swabs	25	7	(28)
Total	208	75	(34.40)

Results

1-Isolation and Identification of *Escherichia coli*

The number of *E. coli* isolated were 90 (28.33%),15 (16.63%) environmental isolates and 75(83.33%) clinical isolates. The environmental isolates of *E.coli* were collected as following : 3(20%) were isolated from each of washing basins and beds,2(13.33%)from each of operation hall, medical equipment and birth auditorium,1(6.66) from each of wards floor and tables (Table 3). While the clinical *E.coli* were isolated as following: 1(1.33%)was obtainedfromburns,7(9.33%)fromHighvaginalswabs,11 (26.82%)fromWounds infections,18(24.00%)from Diarrheal infections and 38 (56.71 %)from Urinary tract infections (Table 4). The identification of *E .coli* isolates was done in accordance to, morphological and biochemical tests. Their colony shape which appeared circular in shape, pink, non viscous colonies, lactose fermentation on MacConkey agar, and metallic sheen onEosin-methylene blue agar. The results of biochemical tests indicated positive for indole, Methyl red, gas production, A/A reaction, no H_2S production in TSI agar, while urease, Vogas-Proskuar and citrate were negative. Identification of *E.coli* were confirmed by using API 20E system. The results showed that all the 90 isolates were *E.coli*.

2-Antibiotic Susceptibility Pattern

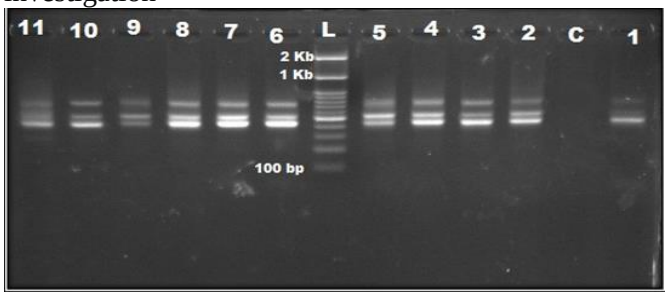
The results showed out of 90 isolates, 83(92.22 %) were multidrug resistant which resistant to a minimum of three classes of the antibiotics tested.The hight percentage of resistant was for cephalothin (91.11%) followed by ampicillin(90.00%) then for piperacillin (85.55%).while the isolates showed low resistance for imipenem (3.33%)followed by amikacin (13.33%)then chloramphenicol (14.44%).(Table5)

Table5: Percentage of antibiotics resistance by *E.coli* against 16 types of antibiotics according to CLSI 2007 (n=90)

Type of antibiotic	No.(%) of Resistant Isolates		No. (%) of Intermediate Isolates		No. (%) of Sensitive Isolates	
	No.	%	No.	%	No.	%
Amikacin (AK)	12	(13.33)	45	(50.00)	33	(36.00)
Ampicillin (AM)	81	(90.00)	3	(3.33)	6	(6.66)
Aztreonam (ATM)	61	(67.77)	19	(21.11)	10	(11.11)
Cefepime (FEP)	43	(47.77)	16	(17.77)	31	(34.44)
Cefotaxime (CTX)	61	(67.77)	19	(21.11)	10	(11.11)
Ceftazidime (CAZ)	65	(72.22)	19	(21.11)	6	(6.66)
Ceftriaxone (CRO)	63	(70.00)	15	(16.66)	12	(13.33)
Cephalothin (KF)	82	(91.11)	7	(7.77)	1	(1.11)
Chloramphenicol(C)	13	(14.44)	9	(10.0)	68	(75.55)
Ciprofloxacin (CIP)	23	(25.55)	37	(41.11)	30	(33.33)
Gentamicin (CN)	45	(50.00)	10	(11.11)	35	(38.88)
Imipenem (IMP)	3	(3.33)	1	(1.11)	86	(95.55)
Nalidixic acid (NA)	54	(60.00)	23	(25.55)	13	(14.44)
Nitrofurantion (F)	29	(32.22)	48	(53.33)	13	(14.44)
Norfloxacin (NOR)	22	(24.44)	16	(17.77)	52	(57.77)
Piperacillin (PRL)	77	(85.55)	3	(3.33)	10	(11.11)

3-Molecular identification of *E. coli* Isolates

This study was conducted to identify *E.coli* by using molecular method targeting ribosomal intergenic spacer analysis (RISA) involves the PCR amplification of the intergenic spacer region (*ITS*) located between the small (16S) and large (23S) subunits of the ribosomal gene. The DNA of all isolates was extracted and purified by using genomic DNA purification kit. The results were detected by electrophoresis on 1.4%agarose gel and exposed to U.V light in which the DNA appeared as compact bands.Universal primers amplified the intergenic spacer region between the 16S and 23S rDNA (*ITS*) indicated that all isolates 90(100%) gave positive results, Multiple interval bands within a lane reflect multiple *ITS* regions of different bacterial species. Figure(1)The *ITS* primer was the primary criterion for defining isolate as *E.coli* in this investigation



Figure(1): Ethidium bromide-stained agarose gel of PCR amplified products from extracted *E. coli* DNA amplified with primers *ITS* F and ITS R. Lane (L), DNA molecular size marker(100-bp ladder): Lanes (1-11) isolates show positive result with *ITS* Primer ,Lane (C), negative control

4- phylogenetic grouping of *E.coli* isolates

Phylogenetic analysis revealed that 90 *E.coli* isolates segregated in phylogenetic group A &D 36 (40.00%) for each,while B2 18 (20.00%) PCR assays of 75 clinical *E.coli* isolates showed that 30 isolates (40%) belonged to A& D for each and 15 isolates (20%) to B2 phylogenetic groups. PCR assays for phylotyping of 15 environmental isolates indicated that the strains distributed in three phylogenetic groups including 6 isolates (40%) in A&D and 3 isolates (20%) in B2 figure (2).

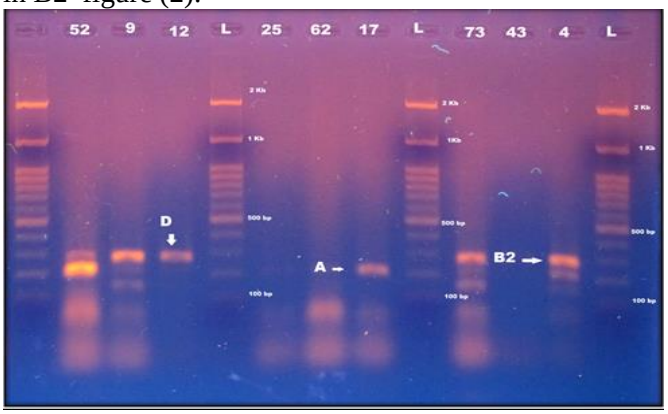


Figure (2): Ethidium bromide-stained agarose gel of PCR amplified products from extracted E. coli DNA amplified with primers chuA, yjaA and TSPE4 .Lanes (L), DNA molecular size marker(100-bp ladder); Lanes (4,43,73) E. coli belong to group B2; Lanes (17,62,25), E. coli belong to group A ;Lanes(12,9,52);E. coli belong n to group D.

Discussion

The intestinal strains of *E. coli* that are not pathogenic, help in the synthesis of essential vitamins like Vitamin K and are essential for digestion. However some strains of *E. coli* are pathogenic and can cause significant morbidity and mortality in humans(Alessandro *et al.*,2006) The diseases caused by pathogenic strains of *E. coli* are classified into three main types. They are enteric or intestinal infections ,neonatal meningitis or neonatal sepsis and urinary tract infections. Physiological studies for *E. coli* have shown the ability of these organisms to adapt themselves to their characteristic habitats(Murthy,2006). *E.coli* accounts for approximately 70 to 95% of community-acquired cases and 50% of all hospital acquired infection(Kucheria et al.,2005). These organisms are responsible for significant social and economic costs for both communities and public chealth resources.

In the present study, The isolation percentage was correspond with many other studies that focused on predominant of E.coli among clinical and hospital environmental samples. In a local study by Al-Hilli(2010), 42(20%) E.coli isolates were obtained from 210 environment samples in Hilla ,Hadi(2008),found that E.coli was the most common (42.6%) organism isolated from patients with significant bacteriuria followed by Klebsiella spp .,AL-Amiedi(2007), noted the most isolate was E.coli (2.55%) isolated from every sterilized Hospital Environment of operation theater ,prematurity wards & emergency ward & kitchenat in Babylone Marternal &Children Hospital, Ali Shah et al.(2003),reported that of the 200 nosocomial Enterobacteriaceae isolates collected from different hospital wards, E.coli was the most frequent (35%) .In another study, out of 387 Enterobacteriaceae isolates collected from six Singapore hospitals, 189(48.9%) were E.coli(Tan et al., 2009). In the United Arab Emirates, of the130 Enterobacteriaceae isolated from patients in six general hospitals, E.coli were predominant 83(63.8%) (Al- Zarouni et al., 2008).In the United States, Jarvis and Martone (1992) reported that E. coli as the most common nosocomial pathogen(13.7%)in surveillance at some hospitals, George et al.(2012) reported E.coli as among the most frequent(16.16%) isolates in hospitals in Kumasi, Ghana. In some hospitals, E. coli ranked first as the most common cause of hospital-acquired infections(Berkley et al.,2005).Also, E.coli strains were found to be the highest and most frequent among the pathogenic microorganisms isolated from ten teaching hospitals in China(Wang et al.,2010). Antimicrobial resistance in bacteria not only in hospitals but also in the community has become an important public health problem(Nugent et al.,2010).In response to these concerns, improving antibiotic prescribing, dose and duration of treatment and as well as monitoring antimicrobial resistance is part of the strategy to reduce antibiotic resistance(Fagon et al.,1994;Davy et al.,2005).The present study revealed that a high percentage of E.coli (92.22 %) were multidrug resistant showing resistance to a minimum of three classes of the antibiotics tested(Falagas and Karageorgopoulos, 2008). In Najaf, Al-Hilali(2010) revealed that a high percentage of *E.coli* isolates (90.9%) were multidrug resistant showing resistance to a minimum of three classes of the antibiotics tested, This percentage was higher in another study conducted by Al-Asady (2009)found that all E. coli isolates (100%) were resistant to at least 12 antibiotics from a total of 26 antibiotics used in his study. Al-Hilli (2010)showed the same ratio (100%) of E.coli isolate obtained from Merjan Teaching Hospital in Hilla city too, were considered as multidrug resistant.High rates of resistance were recorded for Ampicillin (90.00%) and pipracillin(85.55%) Several reports have indicated that these drugs were also less effective against E. coli and other bacterial agents isolated in Najaf (Almohana,2004; Hadi, 2008; Al-Hilali 2010).The present study revealed that all the 90 E.coli isolates were resistant to at least oneofthese β-lactam antibiotics. Siegel(2008)reported that other risk factors may increase resistance to third generation cephalosporins including prolonged antibiotic exposure, severe chronic illness, prior infections, prolonged hospital stay, residence in a long-term care facility, and an indwelling catheter. Levels of resistance are (13.33%) and (50%) against amikacin and gentamicin, respectively.The percentages of aminoglycosides resistance described in present study agree with those reported by Kucukates and Kocazeybek (2002) in Turkey, who found that gentamicin and amikacin were relatively effective against E. coli isolates from various clinical samples. Arora and Devi (2007) found that the resistance to gentamicin and amikacin was detected in (28.89%) and (20.00%) of E. coli isolates ,Also there was a reduce activity of fluoroquinolones including ciprofloxacin and norfloxacin in E.coli isolates (25.55%and 24.44 resistant) comparable with the data from other studies in different parts of the world(El Metwally et al.,2007; carattoli et al., 2005; de Paula and Marin, 2008;Al-Hilali,2010) Who found that all of E.coli isolates were susceptible to these drugs.In agreement with Zamani et al.,(2012) this study demonstrated that (95.55%) E.coli isolates were susceptible to penems class of antibiotics (imipenem).While local studies by Hadi(2008),AI-Hilli (2010)and Al-Hilali(2010), who found that all E.coli isolates were susceptible to imipenems. The bacterial resistance to other non ß-lactam antibiotics were also detected. All isolates showed (32.22%) resistance to nitrofurantoin and (14.44%) to Chloramphenicol. this results disagree with these obtained by Hadi(2008)who found that (52.6%) resistance to nitrofurantoin while(34.2%)resistance to chloramphenicol. Universal primers amplified the intergenic spacer region between the 16S and 23S rDNA genes, indicated that a total of 90(100%)isolates gave positive results with ITS primers that equal to target

product size (539 bp, 436 bp),all these 90 isolates were first confirmed as E. coli by biochemical tests with the API 20 E test. This finding goes in accordance with previous report by Hyatt et al.(2011) In study to identify unknown bacterial species were obtained from the American Type Culture Collection. Determination of E. coli phylogenetic type is being of great epidemiological importanc because there are several reports indicating that there is a potential relationship between E.coli phylogenetic groups, age, sex and disease(Unno et al.,2009).Phylogenetic analyses have grouped E.coli into four main phylogenetic groups A, B1, B2 and D and there is a relation between the phylogeny and the virulence, the virulent extraintestinal strains belong mainly to B2 and to lesser extent to D group, whereas most commensal strains belong to groups A and B1(Desjardins et al., 1995).The reference techniques for phylogenetic grouping are multilocus enzyme electrophoresis and ribotyping. But, these methods are complex and time-consuming(Selander et al.,1986;Bingen et al., 1994;Desjardins et al.,1995).To overcome these drawbacks, Clermont et al.,(2000) described a simple and rapid technique for determination of the phylogenetic group of E. coli strains based on PCR detection of three specific phylogenetic group markers namely chuA, yjaA, and TSPE4C2. In this work PCR results of phylogenetic determination, showed that clinical Isolates mostly fell into group A and D(40 %),followed by B2(20%) while lack of the animal commensal B1 phylogenetic type. However, according to the results environmental isolates mostly belonged to A and D phylogenetic groups(40%)while group B2(20%)and no one isolates belong to B1 phylogenetic types. This result is in line with previous reports indicated that the dominance of A and D phylogenetic type among E. coli isolates in the clinical setting ,Alizade et al.(2013) who indicated that the most E.coli strains belonged to group A(42.2%) followed group D(33.33%) while group B2 (10.4%), Abd ul-Razzaq and Abd ul-Lateef (2011)in Hilla ,who observed that group A was most common isolates(44.4%) although some strains fill into group D(15.5%).while this finding contradicts the previously reported indicated that *E. coli* isolates belonging to phylogenetic group B2 have been shown to predominate in infants with neonatal bacterial meningitis , urinary tract and rectal isolates(Johnson *et al*.,2002; Zhang *et al*.,2002; Bashir *et al.,* 2012) and reflects the increase in the prevalence of the phylogenetic type D and phylogenetic type A among E. coli isolates on the expense of the phylogenetic type B2 in the clinical setting in Thi-Qar. It is not clear whether all E.coli in the intestinal tract of healthy individual at specific time should be considered commensal regardless of their phylogenetic background or only the isolates belonging to groups A and B1(Zhang et al., 2002).Similarly, it is not clear whether E. coli isolates belonging to groups A and B1 from patient with urinary tract infection should be considered as true pathogens or as commensal E. coli that produce infection in a compromised host(Sabate et al., 2006).Distribution of the phylogenetic type populations in E. coli in the clinical setting differ significantly from country to country, from city to another in the same country and most likely from a medical institution to another within the same city as well as from year to year in the same medical institution. Duriez et al.(2001)reported the possible influence of geographic conditions, dietary factors, use of antibiotics, and/or host genetic factors on the distribution of phylogenetic groups among commensal E. coli strains isolated from human stools from three geographically distinct populations in France, Croatia and Mali.

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